

Amendments to the Specification:

Please replace the title of the application (AUTOANTIBODIES SPECIFIC FOR B7-H1) beginning at page 1, line 1 with the following amended title:

--DETECTION OF ANTIBODIES SPECIFIC FOR B7-H1 IN SUBJECTS WITH DISEASES OR PATHOLOGICAL CONDITIONS MEDIATED BY ACTIVATED T CELLS--

Please replace the paragraph beginning at page 14, line 14, with the following amended paragraph:

--Methods of detecting B7-H1-specific antibody in a liquid sample (see above) basically involve contacting a sample suspected of containing B7-H1-specific antibody with a B7-H1 reagent and testing for binding of the B7-H1 reagent to a component of the sample. In such assays the B7-H1 reagent need not be detectably labeled and can be used without a detecting antibody that binds to the B7-H1 reagent. For example, by exploiting the phenomenon of surface plasmon resonance, a B7-H1 reagent bound to an appropriate solid substrate is exposed to the sample. Binding of antibody in the sample to the B7-H1 reagent on the solid substrate results in a change in the intensity of surface plasmon resonance that can be detected qualitatively or quantitatively by an appropriate instrument, e.g., a [[Biacore]] BIACORE® apparatus (Biacore International AB, Rapskatan, Sweden).--

Please replace the paragraph beginning at page 16, line 8, with the following amended paragraph:

Methods of detecting or for quantifying a detectable label depend on the nature of the label and are known in the art. Appropriate labels include, without limitation, radionuclides (e.g., ¹²⁵I, ¹³¹I, ³⁵S, ³H, ³²P, or ¹⁴C), fluorescent moieties (e.g., fluorescein, rhodamine, or phycoerythrin), luminescent moieties (e.g., [[Qdot™]] QDOT™ nanoparticles supplied by the Quantum Dot Corporation, Palo Alto, CA), compounds that absorb light of a defined wavelength, or enzymes (e.g., alkaline phosphatase or horseradish peroxidase). The products of

reactions catalyzed by appropriate enzymes can be, without limitation, fluorescent, luminescent, or radioactive or they may absorb visible or ultraviolet light. Assays using such enzymes for detection are referred to as Enzyme-linked Immunosorbent Assays (ELISA). Examples of detectors include, without limitation, x-ray film, radioactivity counters, scintillation counters, spectrophotometers, colorimeters, fluorometers, luminometers, and densitometers.

Please replace the paragraph beginning at page 17, line 13, with the following amended paragraph:

--The 3-D structure of biological macromolecules (e.g., proteins, nucleic acids, carbohydrates, and lipids) can be determined from data obtained by a variety of methodologies. These methodologies, which have been applied most effectively to the assessment of the 3-D structure of proteins, include: (a) x-ray crystallography; (b) nuclear magnetic resonance (NMR) spectroscopy; (c) analysis of physical distance constraints formed between defined sites on a macromolecule, e.g., intramolecular chemical crosslinks between residues on a protein (e.g., International Patent Application No. PCT/US00/14667, the disclosure of which is incorporated herein by reference in its entirety), and (d) molecular modeling methods based on a knowledge of the primary structure of a protein of interest, e.g., homology modeling techniques, threading algorithms, or *ab initio* structure modeling using computer programs such as [[MONSSTER]] MONSSTER™ (Modeling Of New Structures from Secondary and Tertiary Restraints) (see, e.g., International Application No. PCT/US99/11913, the disclosure of which is incorporated herein by reference in its entirety). Other molecular modeling techniques may also be employed in accordance with this invention [e.g., Cohen et al. (1990) J. Med. Chem. 33: 883-894; Navia et al (1992) Current Opinions in Structural Biology, 2, pp. 202-210; the disclosures of these two articles are incorporated herein by reference in their entirety]. All these methods produce data that are amenable to computer analysis should a computer analysis be desired. Other spectroscopic methods that can also be useful in the method of the invention, but that do not currently provide atomic level structural detail about biomolecules, include circular dichroism and fluorescence and ultraviolet/visible light absorbance spectroscopy. A preferred method of

analysis is x-ray crystallography. Descriptions of this procedure and of NMR spectroscopy are provided below.--

Please replace the paragraph beginning at page 22, line 29, with the following amended paragraph:

--Any available method can be used to construct a 3-D model of a B7-H1 external region of interest from the x-ray crystallographic and/or NMR data, by for example, using a computer as described below. Such a model can be constructed from analytical data points inputted into the computer by an input device and by means of a processor using known software packages, e.g., [[HKL]] HKL™, [[MOSFILM]] MOSFILM™, [[XDS]] XDS™, [[CCP4]] CCP4™, [[SHARP]] SHARP™, [[PHASES]] PHASES™, [[HEAVY]] HEAVY™, [[XPLOR]] XPLOR™, [[TNT]] TNT™, [[NMRCOMPASS]] NMRCOMPASS™, [[NMRPIPE]] NMRPIPE™, [[DIANA]] DIANA™, [[NMRDRAW]] NMRDRAW™, [[FELIX]] FELIX™, [[VNMR]] VNMR™, [[MADIGRAS]] MADIGRAS™, [[QUANTA]] QUANTA™, [[BUSTER]] BUSTER™, [[SOLVE]] SOLVE™, [[O]] O™, [[FRODO]] FRODO™, or [[CHAIN]] CHAIN™. The model constructed from these data can be visualized via an output device of a computer, using available systems, e.g., [[Silicon Graphics]] SILICON GRAPHICS®, [[Evans and Sutherland]] EVANS AND SUTHERLAND™, [[SUN]] SUN™, [[Hewlett Packard]] HEWLETT PACKARD™, [[Apple Macintosh]] APPLE MACINTOSH™, [[DEC]] DEC™, [[IBM]] IBM™, or [[Compaq]] COMPAQ™.--

Please replace the paragraph beginning at page 26, line 18, with the following amended paragraph:

--While not essential, computer-based methods can be used to design the compounds of the invention. Appropriate computer programs include: [[LUDI]] LUDI™ (Biosym Technologies, Inc., San Diego, CA), [[Aladdin]] ALADDIN™ (Daylight Chemical Information Systems, Irvine, CA); and [[LEGEND]] LEGEND™ [Nishibata et al. (1985) J. Med. Chem. 36(20):2921-2928, the disclosure of which is incorporated herein by reference in its entirety].--

Please replace the paragraph beginning at page 37, line 28, with the following amended paragraph:

--Serum samples were obtained from 63 patients with diagnosed RA autoimmune disease and 54 sex- and age-matched healthy donors under the approval of the Internal Review Board of the Mayo Clinic. Diagnosis of RA was defined according to the classification criteria of the American College of Rheumatology. Sera or plasma samples were collected from the 63 RA patients (53 women and 10 men, mean age, 58 years; age range, 17-80 years) and 54 health donors (42 women and 12 men, mean age, 52; age range 20-69). Human IgG was purified by [[ImmunoPure (G)TM]] IMMUNOPURE (G)TM IgG purification kits (Pierce, Rockford, IL).--

Please replace the paragraph beginning at page 38, line 20, with the following amended paragraph:

--BALB/c mice were immunized with purified B7-H1Ig mixed with complete Freund's adjuvant (Sigma, St. Louis, MO) and boosted three times with B7-H1Ig in incomplete Freund's adjuvant. Sera from the mice were collected and their specific binding to hB7-H1 was determined by ELISA and fluorescence flow cytometry (FFC) analysis of 293 cells transfected with and expressing cDNA encoding hB7-H1 (B7-H1/293 cells) [Dong et al. (1999) Nature Med. 5:1365-1369]. Spleen cells from mice with the highest titer of hB7-H1-specific antibody in their sera were fused with SP2/0 myeloma cells to produce hybridoma cells using standard techniques. After several rounds of selection by ELISA and FFC, 2 clones (2H1 and 5H1) producing antibody which consistently stained B7-H1/293 cells were obtained. The isotype of both 2H1 and 5H1 is IgG1. Culture supernatants of the 2H1 and 5H1 hybridomas were concentrated and purified by with [[Protein G -SepharoseTM]] PROTEIN G-SEPHAROSETM columns (Pierce, Rockford, IL) and dialyzed in LPS (lipopolysaccharide)-free PBS. In some experiments, polymyxin B was incorporated in the assays of cell proliferation and cytokine secretion to neutralize residual LPS.--

Please replace the paragraph beginning at page 39, line 5, with the following amended paragraph:

--Freshly isolated human peripheral blood mononuclear cells (PBMC; 1×10^7 cells/ml) were stimulated with 5 μ g/ml of PHA (phytohemagglutinin; Sigma) for various lengths of time. The cells were harvested and analyzed by FFC after 0, 24 and 48 hours of treatment. For direct immunofluorescence staining, T cells were incubated at 4°C with 1 μ g of FITC- or PE-conjugated mAb for 30 minutes and analyzed by FFC using a [[FACScan™]] FACSCAN™ flow cytometer (Becton Dickinson, Mountain View, CA) with Cell Quest software (Becton Dickinson) as described previously [Dong et al. (1999)]. mAb specific for CD4 (RPA-T4), CD8 (RPA-T8), CD45RO (UCHL1) were purchased from BD-PharMingen (San Diego, CA) and rabbit anti-human TRAIL polyclonal antibody was purchased from Alexis Biochemicals (San Diego, CA). For indirect immunofluorescence staining, cells were first incubated with B7-H1-specific mAb (3 μ g/sample) at 4°C for 30 minutes. The cells were washed and further incubated with FITC (fluorescein isothiocyanate)- (Biosource, Camarillo, CA) or PE (phycoerythrin)- (Southern Biotechnology Associates, Inc., Birmingham, AL) conjugated goat anti-mouse IgG F(ab')₂ for 30 minutes at 4°C. Mouse IgG1 (Sigma) was used as control Ig in the indirect staining experiments. In some experiments, cells were treated with human Ig before incubation with FITC- or PE-conjugated mAbs to prevent non-specific binding of antibodies via Fc receptors on the cells.--

Please replace the paragraph beginning at page 40, line 1, with the following amended paragraph:

--To detect interleukin (IL)-10, supernatants were harvested at 24, 48 and 72 hours from the cultures and the concentrations of IL-10 were determined by sandwich ELISA methods (BD-PharMingen) according to manufacturer's instructions. T cell proliferation was determined by the addition of 1.0 μ Ci ³H-TdR (³H-thymidine) 16 hours prior to harvesting of the cultures. Cell proliferation was measured in terms of the amount (in counts per minute; cpm) of ³H-TdR incorporated into the cells. Radioactivity was measured by liquid scintillation counting in a

[[MicroBeta TriLux™]] MICROBETA TRILUX™ liquid scintillation counter (Wallac, Finland).--

Please replace the paragraph beginning at page 40, line 20, with the following amended paragraph:

--Total RNA was prepared using [[TRI Reagent™]] TRI REAGENT™ (Sigma) from 5 x10⁶ T cells which had been stimulated by anti-CD3/B7-H1 mAb or anti-CD3/control Ab for 24, 48, 72 hours. 10 µg of RNA was used as a template for ³²P cDNA probe synthesis. A human Apoptosis1 [[GEArray™]] GEARRAY™ (SuperArray Inc., Bethesda, MD) was used to analyze the expression of 23 apoptosis-related genes and two control "house-keeping" genes, i.e., actin and GAPDH genes. Analysis of gene expression using the Apoptosis1 [[GEArray]] GEARRAY™ was carried out by side-by-side hybridization with the cDNA probes according to the manufacturer's instructions. A STORM™ Phosphoimager system (Molecular Dynamics, Sunnyvale, CA) was used to directly quantify the intensity of the signals. The relative abundance of a particular transcript was estimated by comparing its signal intensity to the signal derived from Beta-actin or GAPDH. Data are expressed as fold increase in signal obtained with cDNA derived from T cells that were stimulated with B7-H1 mAb versus control antibody.--

Please replace the paragraph beginning at page 41, line 3, with the following amended paragraph:

--The [[CaspaTag™ Caspase-3 (DEVD) Activity kit™]] CASPATAG™ CASPASE-3 (DEVD) ACTIVITY KIT™ (Intergen, Purchase, NY) was used to detect the activated form of caspase-3 in CD4+ T cells. The kit detects active caspases in living cells by means of a carboxyfluorescein labeled caspase inhibitor (FAM-DEVD-FMK). The inhibitor irreversibly binds to active caspases and the caspase positive cells were detected by FFC according to the manufacturer's instructions. Briefly, 300 µl of 10⁶ cells/ml was added to a fresh test tube and incubated with 10 µl of 30 x diluted FAM-DEVD-FMK solution for 1 hour at 37 °C under 5% CO₂ in the dark. After incubation, the cells were washed twice with 2 ml of 1 x wash buffer

Applicant : Chen et al.
Serial No. : 10/719,477
Filed : November 21, 2003
Page : 8 of 23

Attorney's Docket No.: 07039-443001 / MMV-02-065

(supplied by the manufacturer of the kit), re-suspended in 400 μ l of 1 x wash buffer, and analyzed by FFC.--